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Title: Different temperature dependence of the bacterial brGDGT isomers in 35 Chinese lake sediments compared to that in soils

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Abstract: During the last decade, the distribution of branched glycerol dialkyl glycerol tetraethers (brGDGTs) in lacustrine sediments has been widely used to reconstruct past variations in lake temperature. A prerequisite for the application of brGDGTs to lacustrine paleoclimate reconstructions is to understand the sources of brGDGTs in lake systems and the processes that influence their distribution. In this study, we investigated the distribution of brGDGTs in core-top sediments from 35 lakes across China, with a broad mean annual air temperature (MAAT) range but a constrained pH range, to explore the effect of temperature. The results reveal a contrasting response of MBT'5ME and MBT'6ME to temperature in lake environments compared to that in soils. The sedimentary distributions of 5- and 6-methyl brGDGTs exhibit different relationships with temperature, with most of the latter being correlated to MAAT while the former responding to temperature by only hexamethylated compounds. In both global and Chinese soils, most 6-methyl brGDGTs have no relationship with MAAT but the distribution of 5-methyl brGDGTs is correlated with MAAT. The different behaviors suggest that both 5- and 6-methyl brGDGTs-producing communities might be different in lakes and soils. In addition, in lakes from cold regions (MAAT < 5 °C), the brGDGT distribution correlates only with warm season temperatures (April to October) but exhibits no correlation with cold seasons, suggesting a seasonal bias in brGDGT production in these lakes. This bias towards the warm season is not found in lakes from warmer regions (MAAT > 5 °C). Based on these results we propose new temperature calibrations for paleotemperature reconstructions in Chinese alkaline lakes.

Highlights

5- and 6-methyl brGDGTs measured in 35 Chinese lakes

Seasonal bias towards warm months in cold region lakes

Different responses to temperature between lakes and soils

Different temperature dependence of the bacterial brGDGT isomers in 35 Chinese lake sediments compared to that in soils

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ABSTRACT

During the last decade, the distribution of branched glycerol dialkyl glycerol tetraethers (brGDGTs) in lacustrine sediments has been widely used to reconstruct past variations in lake temperature. A prerequisite for the application of brGDGTs to lacustrine paleoclimate reconstructions is to understand the sources of brGDGTs in lake systems and the processes that influence their distribution. In this study, we investigated the distribution of brGDGTs in core-top sediments from 35 lakes across China, with a broad mean annual air temperature (MAAT) range, but a constrained pH range, to explore the effect of temperature. The results reveal a contrasting response of MBT'_{5ME} and MBT'_{6ME} to temperature in lake environments compared to that in soils. The sedimentary distributions of 5- and 6-methyl brGDGTs exhibit different relationships with temperature, with most of the latter being correlated to MAAT while the former responding to temperature by only hexamethylated compounds. In both global and Chinese soils, most 6-methyl brGDGTs have no relationship with MAAT but the distribution of 5-methyl brGDGTs is correlated with MAAT. The different behaviors suggest that communities producing 5- or 6-methyl brGDGTs might be different in lakes and soils. In addition, in lakes from cold regions (MAAT < 5 °C), the brGDGT distributions correlate only with warm season temperatures (April to October) but exhibit no correlation with cold seasons, suggesting a seasonal bias in brGDGT production in these lakes. This bias towards the warm season is not found in lakes from warmer regions (MAAT > 5 °C). Based on these results we propose new temperature calibrations for paleotemperature reconstructions in Chinese alkaline lakes.

Keywords: MBT'; isomeric brGDGTs; temperature calibration; lakes; soil

1. Introduction

Lacustrine sediments are useful archives for continental paleoclimate reconstruction, with the preferable preservation of organic matter in lakes being particularly beneficial for the application of organic proxies (Castañeda and Schouten, 2011). One of the most important proxies applied to lacustrine sediments is based on the distribution of branched glycerol dialkyl glycerol tetraethers (brGDGTs, see Fig. 1 for structures) sourced from unknown bacteria (Weijers et al., 2006; Sinninghe Damsté et al., 2011; 2014). The degrees of cyclization and methylation of brGDGTs, expressed as the CBT and MBT indices, are correlated with environmental factors in soils (pH and both pH and mean annual air temperature, MAAT, respectively), which led to the establishment of a quantitative temperature calibration based on MBT-CBT (Weijers et al., 2007). This calibration was later extended and modified by Peterse et al. (2012), yielding the MBT'-CBT index and can also be applied to lacustrine sediments that receive substantial soil inputs.

However, the application of the soil-based calibrations is not straightforward. An increasing number of studies have found evidence for in situ production of brGDGTs in lakes, either in the water column or sediments (e.g., Tierney and Russell, 2009; Blaga et al., 2010; Tierney et al., 2012; Wang et al., 2012; Buckles et al., 2014; Loomis et al., 2014; Weber et al., 2015; Li et al., 2016), and/or seasonal variability in brGDGT production (e.g., Sun et al., 2011; Shanahan et al., 2013; Loomis et al., 2014; Hu et al., 2016). This has led to a variety of lake-specific brGDGT-based temperature calibrations (e.g., Tierney et al., 2010; Zink et al., 2010; Pearson et al., 2011; Sun et al., 2011; Loomis et al., 2012; Foster et al., 2016), and thus necessitates more exhaustive studies of the processes that influence the brGDGT distribution within lakes before their application to paleoclimate reconstruction.

Furthermore, recent work revealed the existence of a series of structural isomers (Fig. 1), the 6-methyl brGDGTs in which methyl groups occur at the ω/α6 position, that co-elute with 5-methyl brGDGTs (with methyl groups at the ω/α5 position) using traditional analytical methods (De Jonge et al., 2013). 6-Methyl brGDGTs are widespread in peat (De Jonge et al., 2013; Naafs et al., 2017b), rivers (De Jonge et al., 2014b), lakes (Weber et al., 2015; Ding et al., 2016) and soils (De Jonge et al., 2014a; Naafs et al., 2017a). Using improved analytical methods it was shown that in both soils and peat, these two isomers exhibit different relationships with environmental factors. In general, the distribution of 5-methyl brGDGTs (represented by the

MBT'$_{5ME}$ index) is correlated to temperature whereas the relative abundance of 6-methyl isomers is correlated to pH in both soils and peat deposits (De Jonge et al., 2014a; Naafs et al., 2017a; 2017b).

However, up to now there are only a few investigations of 5- and 6-methyl isomers in lake systems (De Jonge et al., 2015; Weber et al., 2015; Ding et al., 2016; Dang et al., 2016a; Russell et al., 2018), and the environmental controls (especially temperature control) on these isomers in Chinese lacustrine environments are yet to be deciphered. In particular, it is unknown if the relationships between the 5- and 6-methyl brGDGT isomers with environmental factors are the same in lakes as observed for soils. The difference of the temperature dependence of brGDGTs between soils and lakes has been discussed, but this difference was assigned to in situ production overprinting on the original distribution pattern of brGDGTs (e.g., Tierney and Russell, 2009; Tierney et al., 2010; Loomis et al., 2011; Sun et al., 2011) rather than to the differential temperature response strategies of brGDGT isomers (i.e. 5- and 6-methyl brGDGT isomers).

As many environmental factors would affect brGDGT distributions (e.g., Tierney et al., 2010; Dang et al., 2016a), especially pH, we targeted 35 alkaline lakes in China that span a broad temperature gradient (from –0.2 to 17.2 °C MAAT) to explore the quantitative relationship between brGDGT distributions and temperature. We further compare those Chinese lacustrine distributions and relationships to those from the global soil dataset (De Jonge et al., 2014a) and Chinese soil dataset (Ding et al., 2015; Yang et al., 2015; Lei et al., 2016; Wang et al., 2016); this reveals distinct behaviors for 5- and 6-methyl brGDGT between lakes and soils, recognition of which will be conducive to the development of more accurate temperature calibrations.

2. Materials and methods

2.1. Sampling and environmental parameters

We augment a previously published 17-sample lake brGDGT dataset (Dang et al., 2016a) with an additional 22 new surface sediment samples. Thirty-nine surface sediments were collected from the center of 35 Chinese lakes (Fig. 2; Supplementary

Table S1). All lake surface sediments were obtained using a Peterson MY-051 portable grab sampler and sampling depth was 0 to < 3 cm. Each sample consisted of a homogenized mixture of three subsamples that were collected from each individual lake, wrapped in combusted aluminum foil, and then stored in a sealed bag. All samples were put into incubators with dry ice, transported to the laboratory, and then stored at –20 °C until further analysis.

The MAAT and mean monthly air temperature (MMAT) for the sites of all lakes were obtained from the nearest meteorological station of the Chinese Meteorological Data Sharing Service System, which spans the period from 1970 to 2000. Average values were calculated if the meteorological data consisted of more than one station (Supplementary Table S1). Surface water pH, oxidation-reduction potential (ORP), dissolved oxygen (DO) and conductivity (cond) were measured using a multi-parameter digital analyzer (HQ30d) at the time of sampling (June to September). Each parameter was recorded as an average value of three replicates. The reported depth of each sample was the sampling depth from the water surface and was measured by the grab sampler.

2.2. Lipid extraction

The extraction method followed Dang et al. (2016a). After freeze drying, the samples were ground into powder and ultrasonically extracted with dichloromethane:methanol (9:1, v:v) five times. The total extracts were condensed and base hydrolyzed in 1M KOH/methanol solution (5% H_2O by volume). The neutral fractions were then separated into apolar and polar fractions using silica gel columns. The polar fractions were concentrated and passed through 0.45 μm PTFE syringe filters and dried under N_2. These fractions were stored at –20 °C until analysis.

2.3. GDGT analysis and proxy calculation

BrGDGTs were analyzed using an Agilent 1200 series high performance liquid chromatography–atmospheric pressure chemical ionization-mass spectrometry

(HPLC–APCI-MS). The GDGTs were separated using two silica columns in tandem (150 mm × 2.1 mm, 1.9 μm; Thermo Finnigan, USA), maintained at 40 °C (Yang et al., 2015). The elution gradients were 84% *n*-hexane (A): 16% EtOAc (B) for 5 min, 84/16 to 82/18 A/B for another 60 min, then to 100% B in 21 min and kept for 4 min, followed by a return to 84/16 A/B for 30 min. The flow rate was 0.2 mL/min. The APCI-MS conditions were: vaporizer pressure 60 psi, vaporizer temperature 400 °C, drying gas flow 6 L/min and temperature 200 °C, capillary voltage 3500 V and corona current 5 μA (~3200 V). Selected ion monitoring (SIM) was used, monitoring at *m*/*z* 1050, 1048, 1046, 1036, 1034, 1032, 1022, 1020 and 1018. Each sample was run once and a replicate sample was run between every 10 samples to test the reproducibility.

The CBT and MBT' indices were calculated as the following equations. The roman numerals denote the abundance of corresponding brGDGT structures shown in Fig. 1 (Weijers et al., 2007; Peterse et al., 2012):

$$\mathrm{CBT} = -\log\left[(\mathrm{Ib+IIb+IIb'})/(\mathrm{Ia+IIa+IIa'})\right] \quad (1)$$

$$\mathrm{MBT'} = (\mathrm{Ia+Ib+Ic})/(\mathrm{Ia+Ib+Ic+IIa+IIa'+IIb+IIb'+IIc+IIc'+IIIa+IIIa'}) \quad (2)$$

MBT'_{5ME} and MBT'_{6ME} were based only on either 5- or 6-methyl brGDGTs and calculated as below (De Jonge et al., 2014a):

$$\mathrm{MBT'_{5ME}} = (\mathrm{Ia+Ib+Ic})/(\mathrm{Ia+Ib+Ic+IIa+IIb+IIc+IIIa}) \quad (3)$$

$$\mathrm{MBT'_{6ME}} = (\mathrm{Ia+Ib+Ic})/(\mathrm{Ia+Ib+Ic+IIa'+IIb'+IIc'+IIIa'}) \quad (4)$$

The relative amount of 6- vs. 5-methyl brGDGTs was calculated according to De Jonge et al. (2015):

$$\mathrm{IR_{6ME}} = (\mathrm{IIa'+IIb'+IIc'+IIIa'+IIIb'+IIIc'})/$$
$$(\mathrm{IIa+IIa'+IIb+IIb'+IIc+IIc'+IIIa+IIIa'+IIIb+IIIb'+IIIc+IIIc'}) \quad (5)$$

The fractional abundance of each 5-methyl (or 6-methyl) compound to the combined amounts of 5-methyl (or 6-methyl) brGDGTs and I series-GDGTs was calculated as:

$$[x]_{5ME} = x/(\mathrm{IIIa + IIIb + IIIc + IIa + IIb + IIc + Ia+Ib+Ic}) \quad (6)$$

$$[y]_{6ME} = y/(\mathrm{IIIa'+IIIb'+IIIc'+IIa'+IIb'+IIc'+Ia+Ib+Ic}) \quad (7)$$

The “*x*” denotes individual 5-methyl and I series brGDGTs and the “*y*” represents individual 6-methyl and I series brGDGTs.

2.4. Statistical analysis

Canoco (v. 4.5) software was employed to determine the relationship of environmental factors with the distribution of brGDGTs. The correlation analysis and linear regressions were performed using the SPSS (v. 19.0) software. A p value < 0.05 indicates a significant correlation.

3. Results

3.1. Environmental parameters

Lakes involved in this study spanned a substantial range of mean annual air temperature (MAAT) from –0.2 °C to 17.2 °C, and also wide gradients of oxidation-reduction potential (ORP), dissolved oxygen (DO) and conductivity (Supplementary Table S1). The pH range of these lakes is relatively narrow (7.8 to 9.5), which should enable us to exclude the effect of pH and investigate the relationship between the brGDGT distribution and temperature.

Following previous studies (cf. Tierney et al., 2010), the air temperature was used in this study, because the lake temperatures measured in the field are transient, and the surface water temperature generally tracks the air temperature variation in most lakes (Livingstone et al., 1999; Loomis et al., 2014; Magee et al., 2016). Even in a relatively deep lake with ice cover during winter, both the epilimnetic and hypolimnetic temperatures were correlated with the air temperature (Magee et al., 2016). For these reasons, MAAT was used here for the statistical analysis.

3.2. Distribution of brGDGTs

All the known brGDGTs were present in the surface sediments of the 35 Chinese lakes (Supplementary Table S2). The pentamethylated brGDGTs (i.e. series II brGDGTs) were dominant (49% of the total brGDGTs), followed by hexamethylated (i.e. series III; 32%) and tetramethylated (i.e. series I; 19%) brGDGTs. The 6-methyl brGDGTs dominated in abundance over 5-methyl isomers in 20 of the 35 lakes and the isomer ratio (IR_{6ME}) varied from 0.35 to 0.88. The MBT' index varied between

0.09 and 0.47, and CBT varied between –0.17 and 0.76. In addition, the C5, 6-methyl hexamethylated brGDGTs (III'' isomers; Weber et al., 2015) were found in some of these samples, but appear in only trace amount in most samples. The 7-methyl brGDGTs, initially identified by Ding et al. (2016), can also be observed in almost all samples.

3.3. Temperature dependence of brGDGTs

MBT' exhibits a linear relationship with MAAT (Fig. 3a), but the nature of that relationship differs markedly between lakes with MAAT < 5 °C (cold regions) and those with MAAT > 5 °C (warm regions). The same was observed for MBT'$_{6ME}$ (Fig. 3c). In contrast, MBT'$_{5ME}$ showed no relationship with MAAT (Fig. 3b). In lakes from cold regions, the correlations between both MBT' and MBT'$_{6ME}$ with mean monthly air temperature (MMAT) were significant from April to October, a period when the MMAT is generally above 0 °C, but insignificant from November to March when the MMAT is generally below 0 °C (Table 1). In contrast, in lakes from warm regions, both MBT' and MBT'$_{6ME}$ correlated significantly with MMAT for each month of the whole year (Table 1). To explore whether these two responses could be rationalized, we assumed that the growth temperature is the MAAT for warm-region lakes but the mean April to October temperature for cold-region lakes; although that is a somewhat crude assumption, those average growth temperatures are strongly correlated to MBT'$_{6ME}$ and MBT' across the entire dataset (Fig. 3d, f).

To further evaluate the temperature effect on each GDGT compound, we performed a RDA on fractional abundances of individual brGDGTs from a subset of the lakes from warm regions (n = 27) where most environmental variables are available (Fig. 4). The cumulative percentage variances of the first two axes were 69.8% for the brGDGT distribution data and 97.8% for the relationship between fractional abundances and environmental variables. MAAT primarily loaded on RDA axis 1 which alone explained 66.8% of the brGDGT distributions and 93.7% of the relationship between fractional abundances and environmental variables. The significance test of the forward selection indicated that only MAAT passed the test (*p*

= 0.001), whereas pH (due to the narrow pH range), depth, DO, ORP and conductivity were insignificant factors in affecting brGDGT distributions (*p* = 0.22–0.96), which was also proved by partial RDA results (*p* = 0.19–0.60). In fact, pH was found to show no substantial impact on the cyclization ratios of brGDGTs in high pH lakes (Schoon et al., 2013).

4. Discussion

4.1. Origin of brGDGTs in lacustrine sediments

Because this study focuses on brGDGTs in lake sediments and lacks data on corresponding catchment soils, it is difficult to directly test whether the former derive from in situ production or from surrounding soils via erosion and runoff. However, the distributions of brGDGTs are different in these lakes from global soils (De Jonge et al., 2014a). This can be also observed in other studies focusing on lakes (e.g., Tierney et al., 2010; Zink et al., 2010; Pearson et al., 2011; Sun et al., 2011; Loomis et al., 2012), i.e., a relatively high abundance of III and/or II series of brGDGTs in lakes as opposed to a high abundance of I series of bGDGTs in soils. When comparing the global soil database of De Jonge et al. (2014a) to those in a relatively limited compilation of lakes, IR_{6ME} values are found to partly discriminate lacustrine from soil origins (Fig. 5). This could be further supported by studies of specific lake catchments. For example, mean IR_{6ME} of the soils around Lake Qinghai in northwest China is ~0.80 (Dang et al., 2016b), which is different from that of lake sediments (~0.68). Also, in the watershed of Lake Baikal, the IR_{6ME} is lower in suspended particulate matter than in its inflow river (De Jonge et al., 2015). More importantly, the contrasting behaviors of 5- and 6-methyl brGDGT in our lakes compared to soils (discussed below) further demonstrate that at least some of the brGDGTs are produced in situ.

4.2. The influence of seasonality on brGDGTs in Chinese lakes

On the basis of the sampling design (targeting the alkaline lakes to reduce the covariance of pH), MAAT is the most important environmental variable controlling the brGDGT distributions in the sediment of these alkaline Chinese lakes. The robust relationship between MBT' and MAAT (Fig. 3) also verifies this. However, the linear relationships between MBT' (or MBT'$_{6ME}$) and MAAT are different for lakes from cold and warm regions (Fig. 3), with the former evidently reflecting April to October temperatures (when air temperature is above freezing) and the latter reflecting MAAT (Table 1). Salinity is unlikely to account for the difference between cold and warm lakes because only 3 of 8 cold lakes are saline lakes. The water depth might induce this difference, as most cold lakes in this study are deep-water lakes. The bottom water temperature of deep-water lakes generally keeps near 4 °C all the year round (e.g., Fang and Stefan, 1994; Skowron and Piasecki, 2014). If the water depth was the reason for the lack of correlation between winter temperature and brGDGTs in cold lakes, the brGDGTs should have exhibited no relationship with warm season temperatures as well, but this is not the fact. Moreover, Lake Daihai and Lake Chagan share similar lake depths (~7.9 m and 5.6 m respectively), but have different behaviors. So, the water depth is also unlikely to account for the difference between cold and warm lakes. A possible explanation is the increased seasonal production of brGDGTs in cold lakes, which records the temperatures of warm months. It suggests that the lake GDGT distributions actually reflect growing season temperature; indeed, all 35 lakes are characterized by the same growth temperature vs MBT' (or MBT'$_{6ME}$) relationship (Fig. 3d and 3f). Our finding in Chinese lakes is consistent with many other studies inferring a seasonal bias towards warm months in mid to high latitude lakes (e.g., Pearson et al., 2011; Sun et al., 2011; Shanahan et al., 2013; Foster et al., 2016).

4.3. Differential strategies of bacterial brGDGT methylation in response to temperature between lakes and soils

Numerous studies have shown the difference in temperature calibrations between lakes and soils, and an application of the soil MBT-CBT or MBT'-CBT calibration to lakes will lead to an underestimation of temperature (e.g., Tierney and Russell, 2009; Blaga et al., 2010; Tierney et al., 2010; Zink et al., 2010; Loomis et al., 2011; Sun et al., 2011; Kaiser et al., 2015). This difference was believed to be mainly caused by the different distribution pattern of brGDGTs in lakes and soils, i.e. the in situ production of higher proportions of II and/or III series brGDGTs in lakes (e.g., Tierney and Russell, 2009; Tierney et al., 2012; Buckles et al., 2014; Loomis et al., 2014; Weber et al., 2015), which causes a systematically low MBT. However, the role of 5- and 6-methyl isomers in this difference is unclear, and whether these isomers show a similar behavior in lakes and soils remain unknown.

Our results show that the relationships between the methylation index of 5- and 6-methyl brGDGTs and temperature are different in lake sediments compared to soils and peat. In soils and peat, MBT'_{5ME} is strongly correlated with temperature while MBT'_{6ME} is primarily related to pH (De Jonge et al., 2014a; Yang et al., 2015; Naafs et al., 2017a, 2017b). However, in Chinese lakes, MBT'_{6ME}, rather than MBT'_{5ME}, shows a significant correlation with temperature. This differs from the performance of 5- and 6-methyl brGDGTs in East African lakes, where MBT'_{5ME} strongly correlates with temperature (Russell et al., 2018). This regional difference suggests that local calibration of brGDGT temperature proxy will be more feasible for the reconstruction of temperature than the global calibration.

The aforementioned differences between Chinese lakes and soils are only based on MBT' index, the lack of correlation between MAAT and MBT'_{5ME} in lakes does not mean that the 5-methyl brGDGTs would not respond to temperature. BrGDGTs can be divided into 3 series according to the number of methyl, i.e. the hexamethylated III series (IIIa, IIIb, IIIc and IIIa', IIIb', IIIc'), the pentamethylated II series (IIa, IIb, IIc and IIa', IIb', IIc') and the tetramethylated I series (Ia, Ib and Ic). In Chinese lakes, the relative abundances of the C-5 methylated III series ($III\%_{5ME}$, i.e. the proportion of C-5 methylated III series in the sum of 5-methyl brGDGTs and I series; equation shown in Table 2) and the ratios related to III_{5ME} (i.e. III_{5ME}/II_{5ME} and III_{5ME}/I; equations are shown in Table 2) exhibit significant correlations with temperature (Table 2), while the relative abundances of C-5 methylated II series ($II\%_{5ME}$; equation shown in Table 2) and the ratio of II_{5ME} to tetramethylated compounds (II_{5ME} /I) show weak or no correlation with temperature (Table 2). This suggests that 5-methyl brGDGT-producing bacteria in Chinese lakes respond to temperature solely by regulating the abundance of III_{5ME} series. However, in global or Chinese soils, the II_{5ME} and I series brGDGTs exhibit correlations with MAAT better than the III_{5ME} series brGDGTs (Table 2). Especially, the ratios related to I (II_{5ME} /I and III_{5ME} /I) exhibit moderate correlations with temperature whilst the correlations between III_{5ME}/II_{5ME} and MAAT are relatively weak. This means that the 5-methyl brGDGTs in soils may respond to temperature by changing the relative abundance of II_{5ME} or III_{5ME} to I series. Therefore, the MBT'_{5ME} index, which is mainly governed by variations in the proportion of series I brGDGTs ($I\%_{5ME}$), is sensitive to temperature in soils but is not influenced by MAAT in lacustrine environments.

On the contrary, the 6-methyl brGDGTs in Chinese lakes behave differently from the 5-methyl compounds. The relative abundance of each 6-methyl brGDGT series (i.e. $III\%_{6ME}$ and $II\%_{6ME}$; equations shown in Table 2) and ratios including III_{6ME}/II_{6ME}, III_{6ME}/I and II_{6ME} /I in these lakes correlate significantly with temperature (except $II\%_{6ME}$ in cold regions; Table 2), indicating that the responding mechanism of 6-methyl brGDGTs to temperature may have no selectivity of this compound series. In both global and Chinese soils, however, none of the 6-methyl brGDGT series show a strong correlation with temperature (Table 2).

Overall, 5-methyl brGDGTs may use solely III_{5ME} to respond to temperature in Chinese lakes, while adapt to MAAT by regulating (III_{5ME} + II_{6ME})/I in soils. The 6-methyl bGDGTs may adapt to temperature with no selectivity of compound series (using all series) in Chinese lakes, but do not respond to temperature in soils. The reason for these four different behaviors in response to temperature is still uncertain due to the unknown brGDGT producers. One possible explanation is the brGDGT producers can adapt to temperature via different ways of methylation of 5- and 6-methyl isomers under different environmental conditions, if they can operate such complicated response strategies. However, the structures of 5- and 6-methyl brGDGTs are too similar for the same bacteria to make a difference on the fluidity or stability of cell membranes (De Jonge et al., 2014a). The different performance of 5- and 6-methyl isomers is more likely a result of in the change of the microbial community, and so both of the 5- and 6-methyl brGDGT-producing communities may differ, at least partly, between lakes and soils.

4.4 New temperature calibration for Chinese alkaline lakes

Based on the previous discussion, we developed a new temperature calibration for Chinese alkaline lakes, using a multiple linear regression with the fractional abundance of the compounds that pass the significance test ($p < 0.05$) for the correlation with temperature. The abundances of each compound are calculated based on equations 6 and 7.

$$\text{Growth Temperature} = -29.73 \times [\text{IIIa}]_{5ME} + 91.97 \times [\text{IIIb}]_{5ME} - 551.02 \times [\text{IIIc}]_{5ME} + 22.65 \times [\text{IIb}]_{5ME} + 3.19 \times [\text{Ib}]_{5ME} - 4.23 \times [\text{IIIa'}]_{6ME} - 147.28 \times [\text{IIIb'}]_{6ME} + 460.10 \times [\text{IIIc'}]_{6ME} - 14.59 \times [\text{IIa'}]_{6ME} + 40.02 \times [\text{IIb'}]_{6ME} - 230.78 \times [\text{IIc'}]_{6ME} + 7.54 \times [\text{Ia}]_{6ME} + 29.48 \times [\text{Ic}]_{6ME} + 12.73$$

($r^2 = 0.91$, RMSE = 1.10 °C, n = 39; Fig. 6)

This r^2 and RMSE are improved compared to that of the original MBT' ($r^2 = 0.70$, RMSE = 1.96 °C, n = 39; Fig. 3d) and the MBT'_{6ME} -based calibration ($r^2 = 0.75$, RMSE = 1.78 °C, n = 39; Fig. 3f). However, as the distribution of brGDGTs is also affected by some other environmental factors (Tierney et al., 2010; Dang et al., 2016a), in particular the water pH, more lakes with variable pH are needed in the future for developing a calibration applicable to lakes with a broad range of water pH.

5. Implications and conclusions

The investigation of 35 Chinese alkaline lake sediments further verifies an autochthonous production of brGDGTs in lakes. A seasonal bias towards warm months exists in the Chinese lakes from cold regions, suggesting the application of brGDGT-based calibrations to cold lakes should be treated with caution. After separating 6-methly brGDGTs from the original 5-methyl counterparts, a different response of MBT'_{5ME} and MBT'_{6ME} to temperature in lake environments and soils was identified. When delving deep into the variations of each compound series, four different behaviors of brGDGTs in response to temperature were found in soils and lakes. These different response strategies imply that the brGDGT producers may change the ways of methylation of 5- and 6-methyl isomers depending on the environmental conditions to adapt to temperature or that both 5- and 6-methyl brGDGT-producing communities may be different, partly if not wholly, between lakes and soils. In addition, this study attempts to establish a preliminary temperature calibration for Chinese alkaline lakes, which could help refine the application of brGDGTs to lacustrine palaeoclimate records. We also highlight the importance of separating 5- and 6-methyl isomers and the need of more lacustrine samples in future studies for improving the accuracy of the calibrations.

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Figure and table captions

Fig. 1. The structures of bacterial branched glycerol dialkyl glycerol tetraethers (brGDGTs), adapted from Yang et al. (2015).

Fig. 2. Locations of the 35 Chinese lakes and their nearby meteorological stations.

Fig. 3. Plots of MAAT versus MBT' (a), MBT'_{5ME} (b) and MBT'_{6ME} (c), and of the average growth temperature versus MBT' (d), MBT'_{5ME} (e) and MBT'_{6ME} (f). The growth temperature is the MAAT for warm-region lakes (red dots; n = 27) but is the mean temperature of the period from April to October for cold-region lakes (blue dots; n = 12).

Fig. 4. RDA of the lakes from warm regions showing the relationships of environmental variables with brGDGTs. The conductivity (cond), dissolved oxygen (DO) and oxidation-reduction potential (ORP) are standardized logarithmically.

Fig. 5. IR_{6ME} of lakes compared with that of global soils (grey dots; De Jonge et al., 2014a), adapted from Dang et al. (2016a). The lakes are data in this study (sediments, black dots) and Lake Hinterburg [sediment, triangle; IR_{6ME} value is from Weber et al. (2015) and the pH value is from Blaga et al. (2010)], Lake Baikal (suspended particulate matter, circles; De Jonge et al., 2015) and the average value of 102 Chinese lakes (sediments, cube; Ding et al., 2016). The data without pH values are excluded.

Fig. 6. Scatterplots of (a) residual values and (b) estimated temperature versus measured mean air temperature. Residuals (a) show the offset between measured and calculated temperature values, based on calibration in Section 4.4.

Table 1. Correlation coefficients between MBT'_{6ME} and mean monthly air temperature (MMAT)

Table 2. The correlation coefficients between different indices and temperature, showing different ways of methylation of brGDGTs responding to temperature. The 5-methyl brGDGTs use solely III_{5ME} to respond to temperature in Chinese lakes, but adapt to MAAT by regulating $(III_{5ME} + II_{6ME})/I$ in soils. The 6-methyl bGDGTs adapt to temperature with no selectivity of compound series (using all series) in Chinese lakes, but do not respond to temperature in soils. Soil data without MAAT or ratio values are excluded.

"a" Soil data from De Jonge et al. (2014a) and the data without MAAT values are excluded (n = 237).

"b" Chinese soils (n = 240) from Yang et al. (2015), Ding et al. (2015), Lei et al. (2016) and Wang et al. (2016).

"c" Subset of soils (n = 95) with comparable pH range (pH = 7.8–9.5) of lakes in this study.

Figure 1
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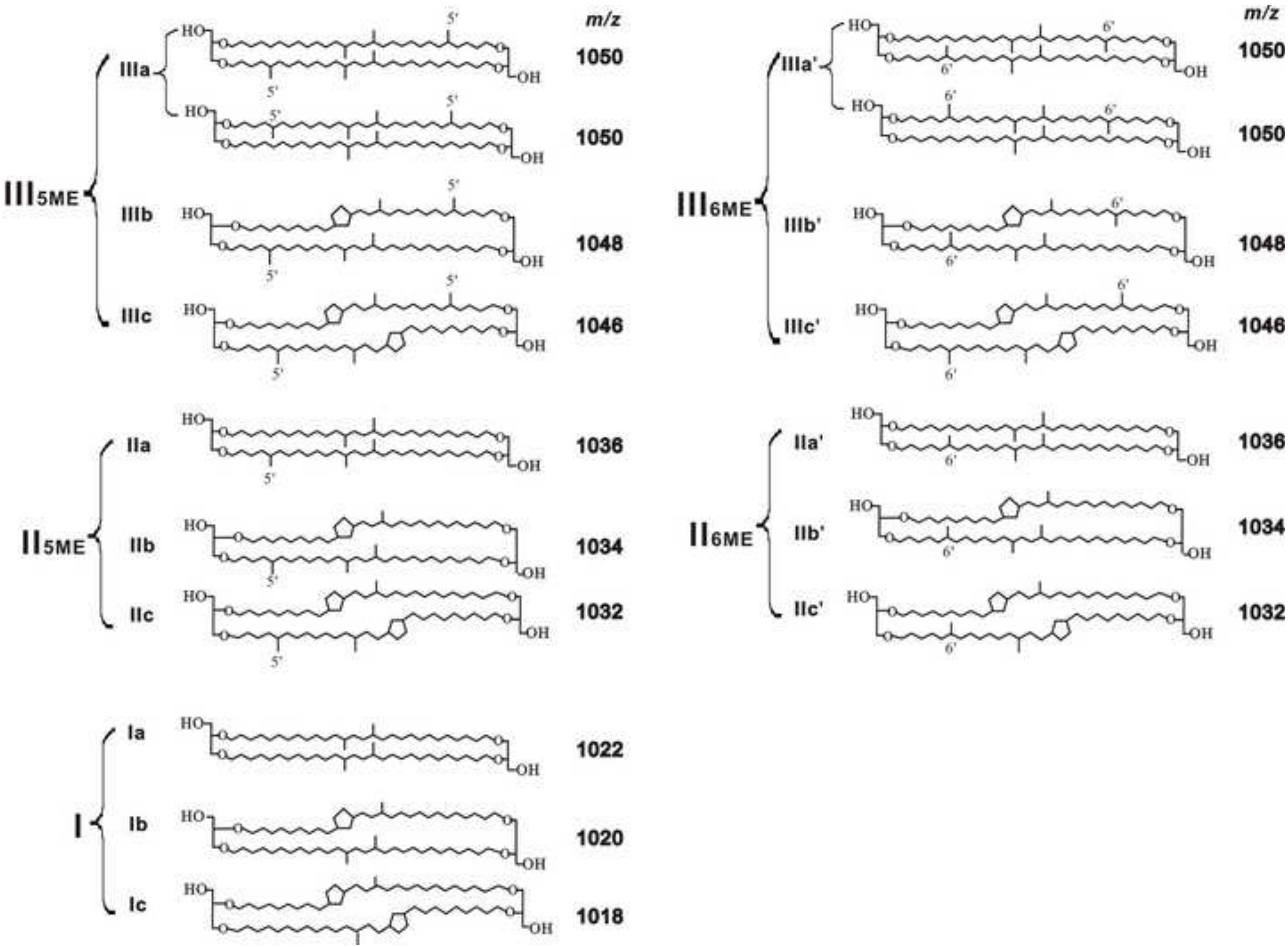

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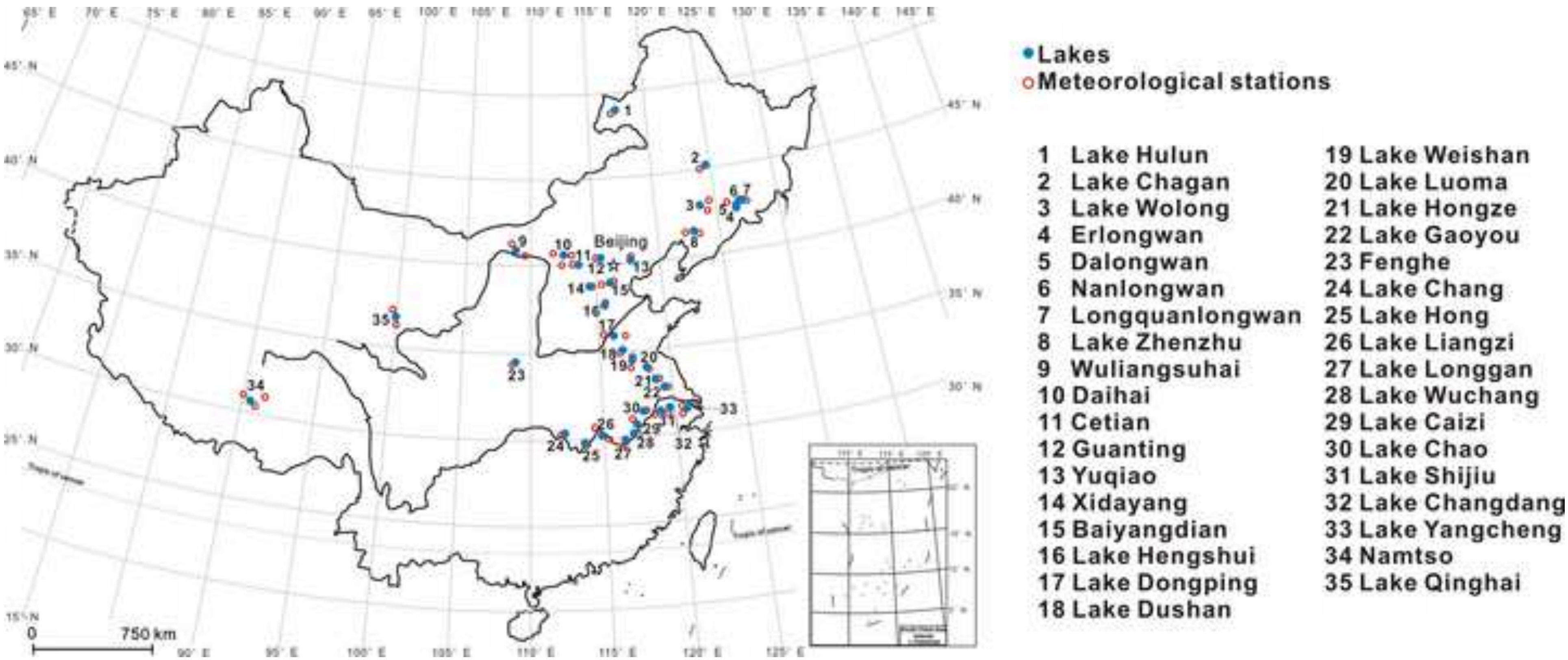

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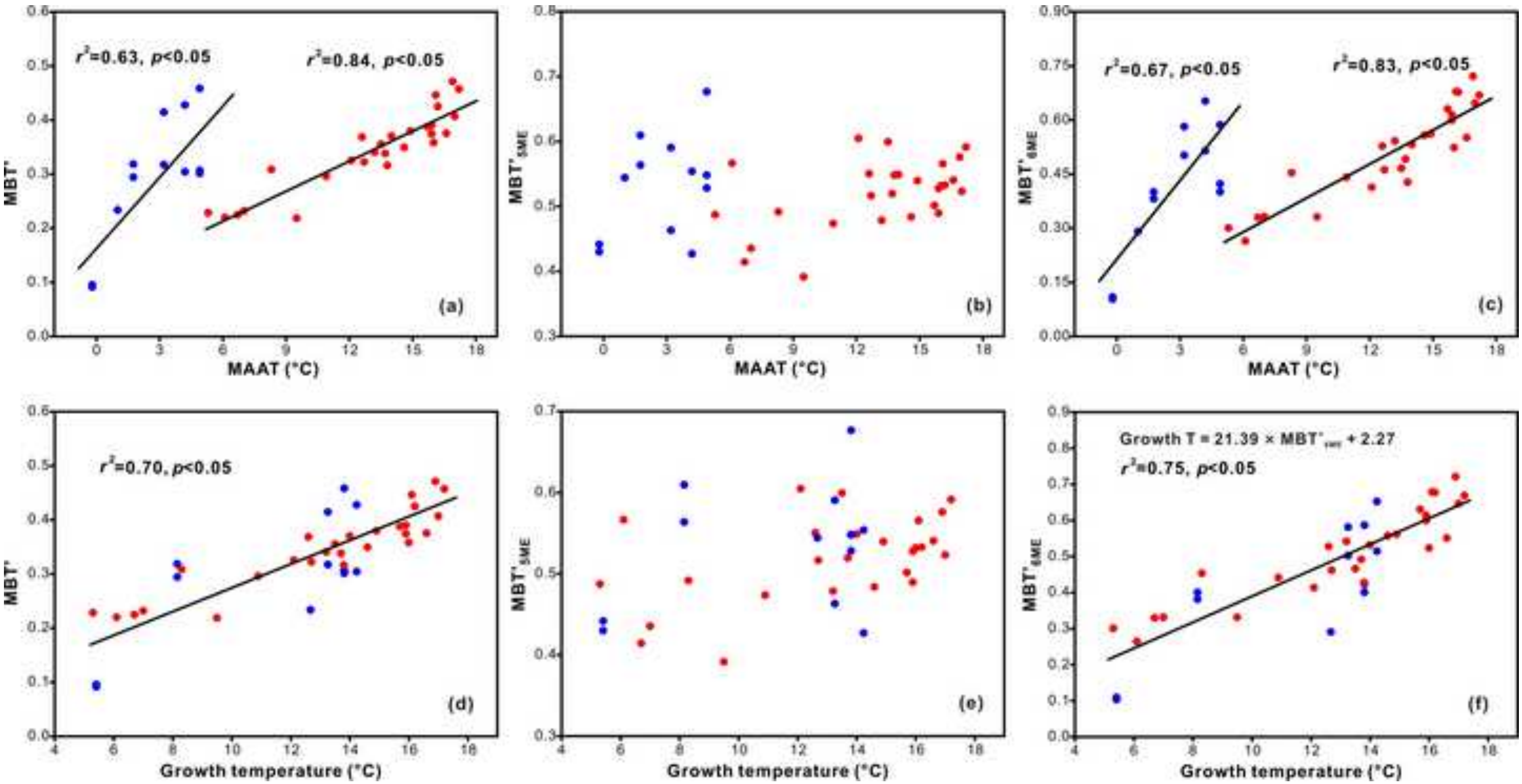

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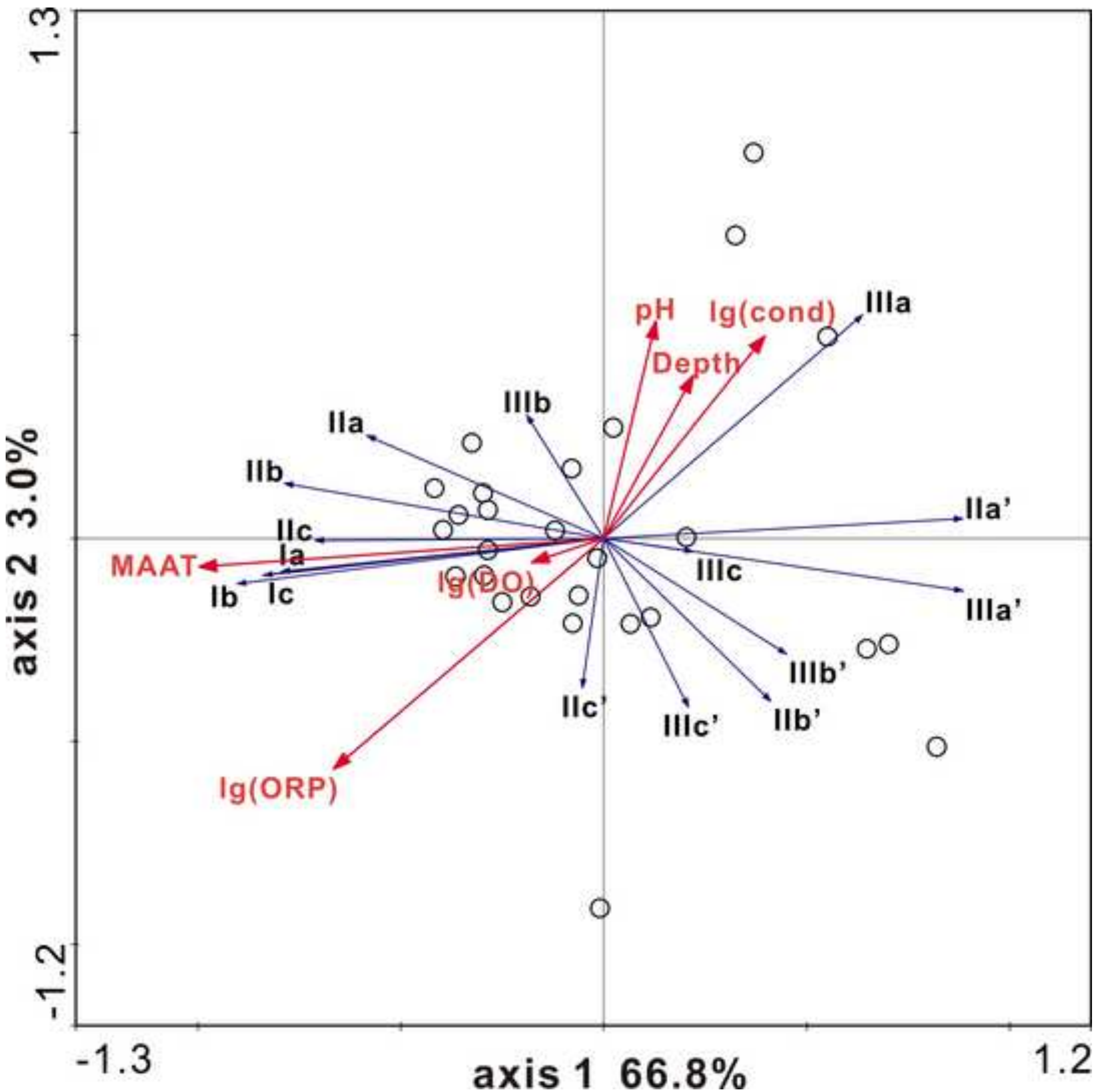

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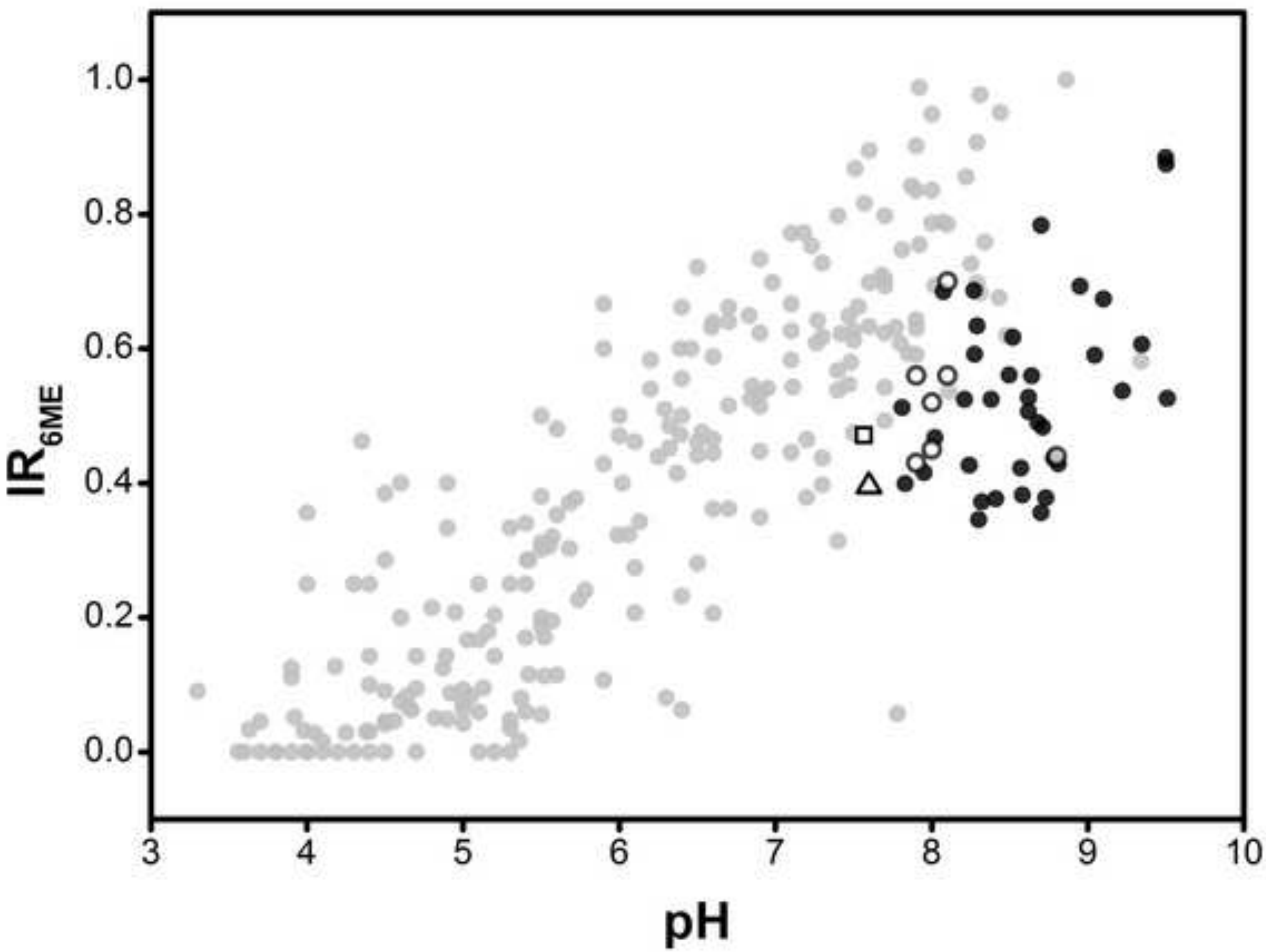

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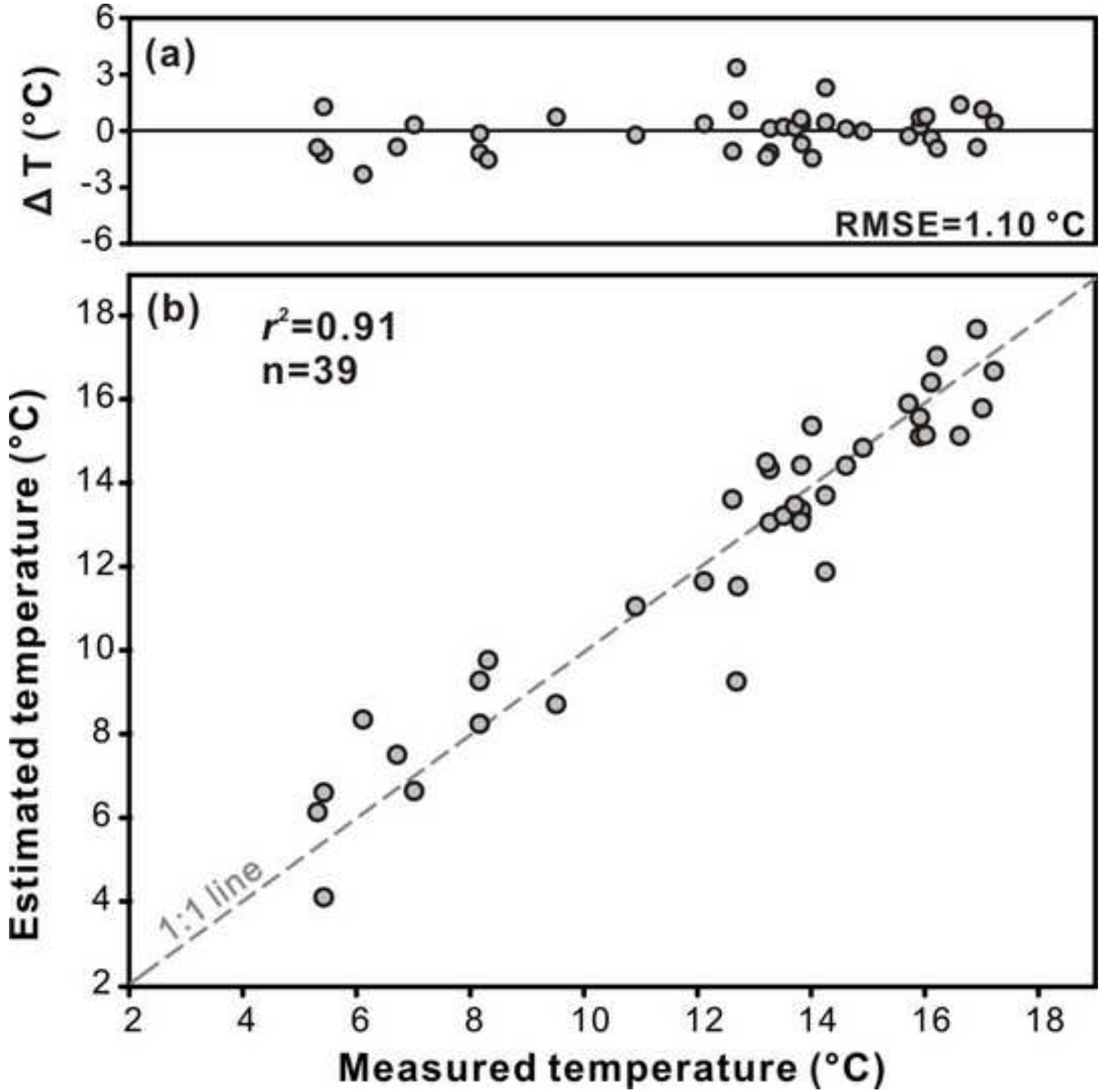

Table 1

			Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
MBT'$_{6ME}$	Cold regions	*r*	-0.41	-0.32	0.33	**0.88**	**0.81**	**0.72**	**0.78**	**0.81**	**0.77**	**0.87**	0.56	-0.22
		p	0.18	0.31	0.30	0.00	0.00	0.008	0.00	0.00	0.00	0.00	0.06	0.48
	Warm regions	*r*	**0.89**	**0.88**	**0.87**	**0.83**	**0.78**	**0.72**	**0.87**	**0.89**	**0.91**	**0.91**	**0.91**	**0.90**
		p	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

The bold type denotes p <0.01

Table 2

				5-methyl brGDGTs						6-methyl brGDGTs				
				III_{5ME}/II_{5ME}	III_{5ME}/I	II_{5ME}/I	$III\%_{5ME}$	$II\%_{5ME}$	$I\%_{5ME}$	III_{6ME}/II_{6ME}	III_{6ME}/I	II_{6ME}/I	$III\%_{6ME}$	$II\%_{6ME}$
Chinese Lakes	Growth T		*r*	-0.85	-0.79	-0.02	-0.85	0.40	0.41	-0.63	-0.58	-0.72	-0.77	-0.72
			p	0.00	0.00	0.89	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00
	MAAT	Cold regions	*r*	-0.85	-0.78	0.03	-0.83	0.38	0.43	-0.78	-0.77	-0.79	-0.83	-0.43
			p	0.00	0.00	0.92	0.00	0.22	0.16	0.00	0.00	0.00	0.00	0.17
		Warm regions	*r*	-0.92	-0.81	-0.21	-0.88	0.26	0.50	-0.58	-0.90	-0.93	-0.87	-0.89
			p	0.00	0.00	0.30	0.00	0.19	0.01	0.00	0.00	0.00	0.00	0.00
Global Soils[a]	MAAT		*r*	-0.44	-0.53	-0.73	-0.63	-0.80	0.81	-0.32	-0.26	-0.26	-0.27	-0.28
			p	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chinese Soils[b]	MAAT		*r*	-0.15	-0.54	-0.79	-0.54	-0.86	0.84	-0.29	-0.15	-0.07	-0.16	0.00
			p	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.28	0.01	0.99
Soils[c] (pH=7.8-9.5)	MAAT		*r*	-0.27	-0.63	-0.66	-0.60	-0.54	0.64	-0.34	-0.51	-0.43	-0.53	-0.09
			p	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.39

III_{5ME} = IIIa+IIIb+IIIc
II_{5ME} = IIa+IIb+IIc
I = Ia+Ib+Ic
III_{6ME} = IIIa'+IIIb'+IIIc'
II_{6ME} = IIa'+IIb'+IIc'
$III\%_{5ME} = III_{5ME}/(III_{5ME}+II_{5ME}+I)$
$II\%_{5ME} = II_{5ME}/(III_{5ME}+II_{5ME}+I)$
$I\%_{5ME} = I/(III_{5ME}+II_{5ME}+I)$
$III\%_{6ME} = III_{6ME}/(III_{6ME}+II_{6ME}+I)$
$II\%_{6ME} = II_{6ME}/(III_{6ME}+II_{6ME}+I)$
$I\%_{6ME} = I/(III_{6ME}+II_{6ME}+I)$

*Response to Reviewers

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Thank you for the evaluation of the manuscript. Careful revision was made in the text, tables and supplemental materials on the basis of your comments. Point-by-point reply was shown below.

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Figure 2

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